REVIEW

Preclinical Characterization of A-582941: A Novel α7 Neuronal Nicotinic Receptor Agonist with Broad Spectrum Cognition-Enhancing Properties

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Among the diverse sets of nicotinic acetylcholine receptors (nAChRs), the α7 subtype is highly expressed in the hippocampus and cortex and is thought to play important roles in a variety of cognitive processes. In this review, we describe the properties of a novel biaryl diamine α7 nAChR agonist, A-582941. A-582941 was found to exhibit high-affinity binding and partial agonism at α7 nAChRs, with acceptable pharmacokinetic properties and excellent distribution to the central nervous system (CNS). *In vitro* and *in vivo* studies indicated that A-582941 activates signaling pathways known to be involved in cognitive function such as ERK1/2 and CREB phosphorylation. A-582941 enhanced cognitive performance in behavioral models that capture domains of working memory, short-term recognition memory, memory consolidation, and sensory gating deficit. A-582941 exhibited a benign secondary pharmacodynamic and tolerability profile as assessed in a battery of assays of cardiovascular, gastrointestinal, and CNS function. The studies summarized in this review collectively provide preclinical validation that α7 nAChR agonism offers a mechanism with potential to improve cognitive deficits associated with various neurodegenerative and psychiatric disorders.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are a family of pentameric ligand-gated ion channels derived from multiple α (*α2–α10*) and β (*β2–β4*) subunit genes (Gotti and Clementi 2004). Multiple, functionally distinct nAChR complexes can be assembled either as homomeric pentamers, as in the case of α7 (Couturier et al. 1990), or as heteropentamers with at least two different subunits, for example, α4β2 nAChRs (Gotti et al. 2006). Among the diverse nAChRs, the role of α7 nAChR subtype in the central nervous system (CNS) has been widely studied (Jensen et al. 2005; Romanelli et al. 2007). This receptor activates and desensitizes rapidly, and exhibits higher Ca^{2+} permeability relative to other nAChR combinations (Dajas-Bailador and Wonnacott 2004). The functional significance of α7 nAChR has been attributed not only to its electrogenic

properties (i.e., modulation of neuronal excitability and neurotransmitter release), but also to its high Ca^{2+} permeability and association with biochemical signaling pathways (Berg and Conroy 2002; Dajas-Bailador and Wonnacott 2004; Role and Berg 1996).

In the brain, the α7 subunit is expressed at high levels in regions involved in learning and memory, particularly the hippocampus and cerebral cortex (Seguela et al. 1993). Although widely distributed across all cortical regions, α7 receptors are located more densely in the cingulate cortex than in the temporal and frontal cortex (Marutle et al. 2001). These receptors are localized at pre- and postsynaptic levels on GABAergic, glutamatergic, and cholinergic neurons, and modulate the release of multiple neurotransmitters, including glutamate, acetylcholine (ACh), and GABA (Barik and Wonnacott 2006; Endo et al. 2005; MacDermott et al. 1999; Wonnacott 1997). Activation of the α7 nAChR results in a localized increase in Ca^{2+} concentration, triggering subsequent activation of additional Ca^{2+} entry mechanisms, including voltage-dependent Ca^{2+} channels (Berg and Conroy 2002; Sharma and Vijayaraghavan 2001). In addition to stimulating neurotransmitter release, activation of α7 nAChRs and associated calcium transients increase the phosphorylation of extracellular receptor kinase (ERK) leading to phosphorylation and recruitment of transcription factors such as cyclic AMP response element-binding protein (CREB). The phosphorylation of CREB can increase the transcription of various genes that may be responsible for long-lasting effects on synaptic plasticity and memory (Adams and Sweatt 2002).

The α7 gene has been implicated as a genetic factor contributing to schizophrenia, in particular, for the auditory sensory processing deficit found in this disease. Diminished inhibition of the P50-evoked response to repeated auditory stimuli has been linked to mutations in chromosome 15q14 locus, with single nucleotide polymorphisms in the promoter of the nAchR gene (Gault et al. 1998; Leonard et al. 2002; Raux et al. 2002). Analysis of knockout or antisense-treated animals has demonstrated that α7 nAChRs play important roles in certain cognitive and attentive tasks (Wehner et al. 2004; Curzon et al. 2006). The α7 nAChR knockout mice are viable and fertile, and lack α-bungarotoxin-binding sites and nicotine-induced fast desensitizing currents in hippocampal GABAergic neurons (Picciotto et al. 2001). Mice that lack the α7 receptor gene show deficits in cognition, including areas of attention and working/episodic memory (Fernandes et al. 2006; Hoyle et al. 2006; Young et al. 2007). It has been demonstrated that these animals acquire the 5-choice serial reaction time task (measure of sustained attention) more slowly than their wild-type littermates indicating that α7 nAChRs are involved in attention processes (Hoyle et al. 2006; Young et al. 2007).

Pharmacological studies have suggested that augmenting α7 nAChR function by subtype selective agonists or positive allosteric modulators is capable of ameliorating the cognitive deficits associated with neuropsychiatric diseases. Thus, over the past decade, a variety of largely quinuclidine pharmacophore-based agonists of α7 nAChRs have emerged, including AR-R 17779 (Mullen et al. 2000; Van Kampen et al. 2004), PNU-282987 (Bodnar et al. 2005), PHA-543613 (Wishka et al. 2006), W-56203 (Tatsumi et al. 2006), and ABBF (Boess et al. 2007). Our lead optimization efforts and structure–activity relationship studies focused on identification of compounds with improved CNS penetration and pharmacokinetic properties relative to some of the quinuclidine α7 nAChR agonists. The present review describes the pharmacological and preclinical tolerability profile of A-582941, a prototype from the biaryl diamine series (Basha et al. 2005). As summarized below, A-582941 shows excellent CNS penetration and exhibits broad-spectrum efficacy in behavioral models of cognition and sensory gating.

Preparation and Physical Properties

A-582941 (2-methyl-5-[6-phenylpyridazin-3-yl]octahydropyrrolo[3,4-c]pyrrole) was synthesized as outlined in figure 1. The bicyclic diamine, **2**, was assembled by dipolar cycloaddition of maleimide with the azomethine ylide formed *in situ* from **1** (Padwa and Dent 1989). Reduction of the imide with $LiAlH_4$, followed by treatment with di-t-butyl dicarbonate provided the N-Boc derivative, **3**. Catalytic hydrogenation effected debenzylation to afford the Boc-diamine, **4**. This entire sequence was carried out on 100-g scale with only a single filtration-type chromatographic purification.

Coupling of the diamine, **4**, with 3-chloro-6-phenylpyridazine was best accomplished by heating equimolar amounts of the reactants at 105 °C in a 1:1 mixture of DMSO and ethyldiisopropylamine for 48 h. The crude product, precipitated directly from the warm reaction mixture by dilution with water, was isolated by filtration and washed with ether to provide material of sufficient purity for the next step. This was heated with formalin in formic acid to remove the Boc group with reductive methylation in a single pot. After removal of most of the formic acid under vacuum, the residue was dissolved in water and made basic (pH∼10) to

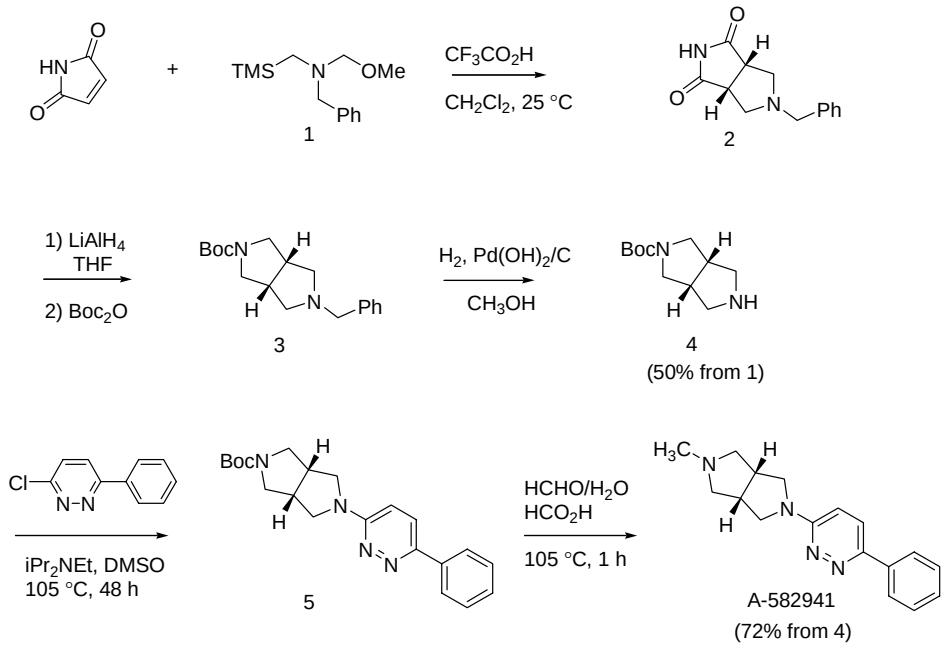

Figure 1 Synthetic route for preparation of A-582941 on multigram scale.

precipitate the crude free base, which was converted to dihydrochloride salt in EtOAc-EtOH. Recrystallization from isopropanol afforded A-582941 as a white, crystalline hydrate in 72%-yield from **4** on 40-g scale.

A-582941 exhibits favorable physical properties for a CNS-active drug. With a low molecular weight (280 Da for the free base) and moderate lipophilicity (ClogP = 2.3), A-582941 has a relatively compact structure with few rotatable bonds, factors considered to facilitate diffusion across the blood–brain barrier (BBB) (Pajouhesh and Lenz 2005). Good permeability ($P_{appA\rightarrow B} = 14.02 \times 10^{-6}$ cm/s) across Caco-2 cells is consistent with excellent absorption potential for A-582941. The molecule possesses two basic sites, the N-methylated tertiary amine and the aminopyridazine, with pK_a values of 8.75 and 4.44, respectively. At physiological pH, the molecule exists substantially in the monoprotonated state, accounting for its good water solubility (10 mg/mL) at pH<8. While the ClogP for the neutral molecule is 2.3, the logD at pH 7.4 was determined to be 1.0, reflecting the greater hydrophilicity of the protonated form. Plasma protein binding of A-582941 was measured by centrifugation of 5-μM solutions in plasma, followed by quantitation of unbound drug in the supernatant. Similarly, moderate plasma protein binding across a range of species (ferret 65%, mouse 77%, rat 72%, dog 70%, monkey 68%, and human 73%) indicated that circulating A-582941 was substantially available for pharmacological action.

In Vitro Pharmacology

Radioligand Binding

Competition binding with the α7 nAChR agonist radioligand [^{3}H]A-585539 in rat brain membranes yielded a K_i value of 10.8 nM (Anderson et al. 2008; Bitner et al. 2007). Nearly equivalent binding affinity (K_i = 17 nM) was observed in membranes from human frontal cortex, indicating that cross-species differences appear to be minimal for this α7 ligand. A-582941 also displaced the α7-selective antagonist [^{3}H]methyllycaconitine (MLA) from rat brain membranes with a K_i value of 88 nM, which is some 8-fold lower than the affinity measured using the agonist radioligand [^{3}H]A-585539, a difference in line with that observed for other α7 nAChR ligands (Anderson et al. 2008).

In contrast, A-582941 has much lower affinity for heteromeric nAChR subtypes, in particular the "high-affinity" $\alpha4\beta2$ subtype measured using [^{3}H]cystine binding to rat brain membranes (K_i >100,000 nM) and ganglionic $\alpha3\beta4^*$ nAChRs measured with [^{3}H]epibatidine binding to IMR-32 cell membranes (K_i = 4700 nM) and the $\alpha1\beta1\gamma\delta$ (neuromuscular junction) receptor (K_i >30,000 nM). In addition, A-582941 was screened for activity across a panel of 78 receptor targets including G-protein-coupled receptors, ligand- and voltage-gated ion channels, and neurotransmitter uptake sites. Significant affinity (>75%

binding at 10,000 nM) was observed only at the $5HT_3$ receptor, an ion channel that has previously been shown to exhibit ligand cross-reactivity with the α7 nAChR. For example, tropisetron, a classical $5HT_3$ antagonist, is equally potent as a ligand at α7 (Macor et al. 2001), and the α7 agonist PSAB-OFP also exhibits high-affinity binding at $5HT_3$ (Broad et al. 2002). In the present case, A-582941 displaces [^{3}H]BRL-43694 binding to human $5HT_3$ receptor with K_i value of 150 nM, representing approximately 15-fold selectivity for the human α7 nAChR relative to $5HT_3$.

Functional Activity

When exposed to *Xenopus* oocytes expressing the recombinant human α7 nAChR, A-582941 evoked rapidly desensitizing currents characteristic of α7 activation. The response was blocked in the presence of the α7 antagonist methyllycaconitine (MLA, 100 nM), and restored after washout of the antagonist. Based on analysis of the evoked concentration-dependent peak currents, the compound was determined to be a partial agonist at human α7, with EC_{50} value of 4260 nM and 52% maximal response compared to a maximally efficacious concentration of ACh (10 mM). Consistent with minimal cross-species differences in binding affinities, A-582941 was also a partial agonist at recombinant rat α7 nAChR, with EC_{50} = 2450 nM and 60% maximal response relative to ACh.

The effects of A-582941 on α7 nAChRs were potentiated by selective positive allosteric modulation. When oocytes were incubated with the α7 nAChR-positive allosteric modulator PNU-120596 (3 μM) (Hurst et al. 2005) followed by determination of the concentration response curve to A-582941, the concentration–activation curves revealed increased amplitudes of the peak ACh–evoked currents (efficacy), leftward shift in the concentration–activation curve, and increased steepness of the slope of the concentration–response curve (Fig. 2A). In the presence of 3000 nM PNU-120596, A-582941 activated human α7 nAChRs with an apparent EC_{50} = 580 nM and 207% efficacy relative to ACh (in the absence of the modulator). In native brain slice preparations, GABAergic spontaneous inhibitory postsynaptic potentials (IPSCs) were recorded from dentate gyrus granule cells of rat brain by whole-cell patch-clamp

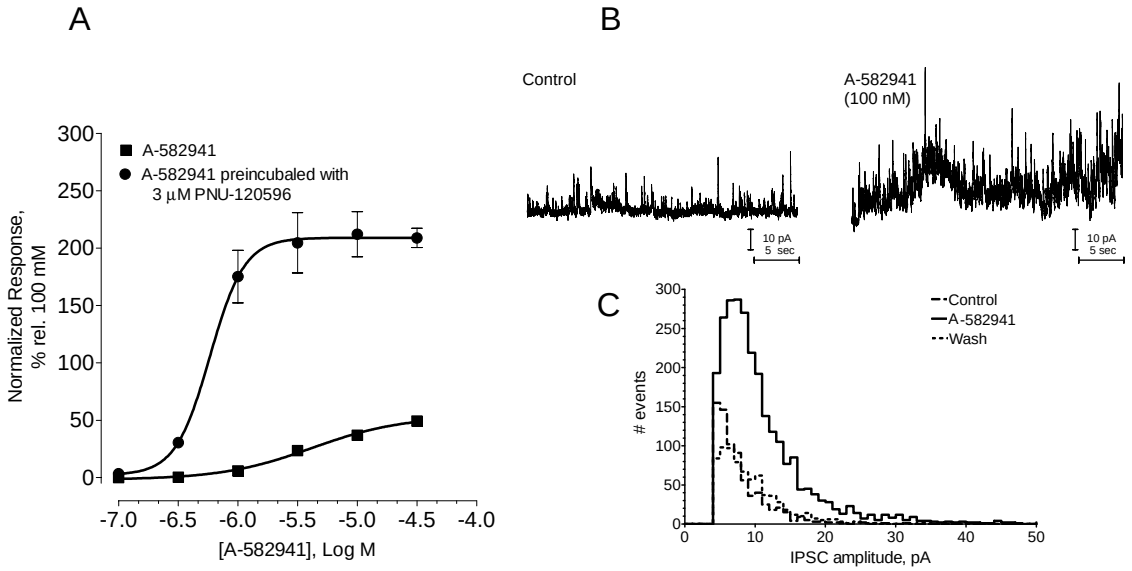

Figure 2 (A) Enhancement of A-582941-evoked responses by PNU-120596. Shown are responses to varying concentrations of A-582941 in the presence or absence of PNU-120596 (3 μM) in *Xenopus* oocytes expressing α7 nAChR. Data are normalized to that evoked by 1 mmol ACh. (B) Effect of A-582941 on GABAergic spontaneous inhibitory postsynaptic potentials (IPSCs) recorded from dentate gyrus granule cells. Experiments were performed where the chloride content of the internal solution was reduced to 13 mM (methanesulfonate substitution) so that the $GABA_A$ chloride current reversal potential was −60 mV, while the glutamate receptor cation current reversed near 0 mV. To record IPSCs, the holding potential was set to 0 mV, minimizing glutamatergic currents and revealing GABAergic IPSCs as positive (upward) deflections. Traces are from one cell exposed to 10 μM PNU-120596 alone (control) and subsequently 100 nM A-582941 in the presence of PNU-120596. Similar results were obtained in 7 other cells and recorded using pClamp9 (Axon Instruments, Union City, CA, USA) in 5-min epochs before (control), during, and after (wash) exposure to A-582941 by bath perfusion. IPSC events during each epoch were detected off-line using MiniAnalysis software (Synaptosoft, Inc., Fort Lee, NJ, USA). (C) Shows the number of events detected in each epoch plotted according to amplitude bin, demonstrating an increase in events across essentially all amplitudes coincident with the administration of A-582941.

as a measure of α7 nAChR activation. Addition of PNU-120596 (10 μM) alone had no effect on the frequency or amplitude of IPSCs. However, subsequent addition of A-582941 (100 nM; Fig. 2B) evoked substantial increases in IPSC activity. To analyze the data, the amplitudes (Fig. 2C) and areas (integrals) of all IPSCs detected in each 5-min epoch were summed together, and the effect of A-582941 measured as a percent increase over the preceding control. A-582941 (100 nM) increased the number of IPSCs by 260 $\pm$ 70% ($n = 8$, $p < 0.05$), the sum of amplitudes by 220 $\pm$ 30% ($p < 0.01$), and the sum of areas by 210 $\pm$ 40% ($p < 0.01$). These responses were reversible upon washout of A-582941.

In accord with its low binding affinity at heteromeric nAChRs, A-582941 did not activate recombinant heteromeric nAChRs ($\alpha4\beta2$, $\alpha3\beta2$, $\alpha3\beta4$, or $\alpha4\beta4$), as measured by Ca^{2+} dynamics in FLIPR. Likewise, it also failed to produce an agonist effect (less than 20% efficacy at concentrations up to 100,000 nM) in the native human $\alpha3\beta4^*$ nAChRs expressed in IMR-32 cells. In *Xenopus* oocytes expressing the $\alpha9\alpha10$ nAChR construct, A-582941 failed to evoke currents up to a concentration of 100,000 nM(D Bertrand, personal communication). Thus, with respect to nAChRs, A-582941 is a highly selective partial agonist, activating only the homomeric α7 subtype. A-582941 also exhibited agonist activity at the human $5HT_3$ receptor, with EC_{50} = 4600 nM (efficacy ~100% relative to 5HT). However, as noted below, the observation that efficacious plasma levels across a range of *in vivo* models approximates the α7 binding K_i values, and especially the observation that that *in vitro* and *in vivo*, biochemical and behavioral effects were blocked or reversed in presence of the α7 nAChR antagonist MLA indicates that the effects of A-582941 are mediated principally via α7 nAChRs.

Biochemical Effects

One of the key phosphorylation cascades involved in learning and memory is the mitogen-activated protein (MAP) kinase pathway, specifically, phospho extracellular-signal regulated kinase (pERK). pERK is increased in brain regions including hippocampus following long-term memory consolidation, and pharmacological inhibition of pERK prevents long-term memory formation in rodent learning paradigms. Specifically, α7 nAChR agonists have been shown to trigger phosphorylation and activation of ERK1/2 (Gubbins et al. 2006), which in turn is critical to several forms of memory, including LTP, fear conditioning, spatial memory, inhibitory avoidance, and object recognition, as well as neuroprotection (Toborek et al. 2007). PC12 cells that expressed α7 nAChRs were used to assess effects on ERK phosphorylation and responses were measured in the presence of PNU-120596, so as to slow receptor desensitization (Hurst et al. 2005). A-582941 increased ERK1/2 phosphorylation with an EC_{50} value of 95 nM, an effect that was attenuated by coincubation with 50 nM MLA.

In vivo effects on MAP kinase/ERK signaling were measured in mice. Administration of A-582941 (0.01–1.00 μmol/kg, i.p.) produced a dose-dependent increase in ERK1/2 phosphorylation in the cingulate cortex and hippocampus (dentate gyrus) within 15 min following administration, as determined by immunohistochemistry. Similar to ERK1/2, increased cAMP response element-binding protein (CREB) phosphorylation was observed in the cingulate cortex 15 min after dosing with A-582941 (0.01, 0.10, and 1.00 μmol/kg, i.p.) (Bitner et al. 2007). Importantly, the dose-dependent increases in ERK and CREB phosphorylation correspond to efficacy in cognition models at the same doses and drug exposures, as described below.

Neuroprotective Properties

α7 nAChRs have been previously implicated in the neuroprotective effects of nicotine. *In vitro* studies using PC12 cells showed that A-582941 (0.1–100 μM) protected against cell death induced by NGF withdrawal. Possible mechanisms that underlie neuroprotective effects of α7 nAChR agonists may include the cell survival signaling pathway, namely, PI3K/Akt/GSK3β (Gubbins et al. 2006). We therefore assessed the effect of A-582941 on the tau kinase, GSK-3β, by assessing the phosphorylation of Ser-9 that leads to its inhibition. Systemic administration of A-582941 (0.1 and 1.0 μmol/kg, i.p.) evoked dose-dependent increases in Ser-9 GSK-3β phosphorylation in the mouse cingulate cortex (Bitner et al. 2007). This inhibition of this predominant tau kinase may underlie, in part, the neuroprotective effects of A-582941, and may contribute to slowing disease progression in various tauopathies.

Microdialysis/*In Vivo* ACh Release

Stimulation of ACh release plays a critical role in learning and memory, and degeneration of cholinergic neurons is closely associated with cognitive effects in Alzheimer's disease (Kasa et al. 1997). ACh release by nicotine has been demonstrated in hippocampus and cortical regions, where α7 nAChRs are abundantly expressed. Nicotine-evoked ACh release in hippocampus, however, does not appear to be mediated by α7, since it could not be blocked by selective antagonists (Tani et al. 1998; Wonnacott 1997), while the α7 partial agonist GTS-21 (DMXB) had no effect on hippocampal ACh levels (Tani et al.

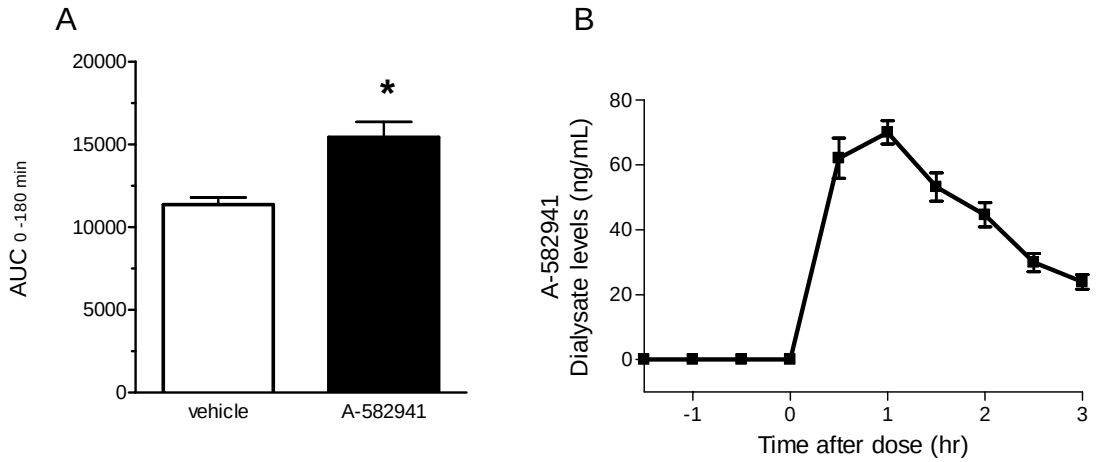

Figure 3 Microdialysis studies with A-582941. (A) Effect of A-582941 on acetylcholine release in the medial prefrontal cortex of freely moving rats. Five animals per treatment group received A-582941 (3 μmol/kg, i.p.) repeated for 3 days, once daily, whereas control animals received only sterile water. After drug administration, microdialysate samples were analyzed for extracellular levels of acetylcholine by HPLC with electrochemical detection. Shown is mean $\pm$ S.E.M. area under the curve (AUC in arbitrary units, 0–180 min) after administration of A-582941 collapsed from days 1–3; $n = 5$ per group. $^*P < 0.05$ A-582941 (3 μmol/kg) versus vehicle-treated animals. (B) Recovery of A-582941 from a microdialysis probe in the medial prefrontal cortex of freely moving Sprague-Dawley rats ($n = 5$) following systemic administration of drug (3 μmol/kg, i.p.). Extracellular brain levels are extrapolated from the dialysate levels using an estimated probe *in vitro* recovery of 5.9–8.4% for A-582941.

1998). Cortical release of ACh is also induced by nicotine (Quirion et al. 1994), but the involvement of α7 nAChR had not been addressed. Therefore, the effect of the selective α7 agonist A-582941 on ACh release was evaluated by *in vivo* microdialysis. Systemic administration of A-582941 (3 μmol/kg, i.p.) led to a moderate increase in ACh release in the medial prefrontal cortex (mPFCx) of freely moving rats, an effect that remained stable after the second and third administration of the drug on consecutive days (Fig. 3A). This dose is larger than that required for efficacy in cognition models, so the relevance of the observed ACh effect is not entirely clear. Nevertheless, the experiment demonstrates that ACh release in mPFCx can be mediated by α7 receptors. In this context, it is worth noting that the α7 partial agonist SSR180711 was recently shown to enhance release of dopamine and ACh release in prefrontal cortex of rat, with effects on ACh release in hippocampus at higher doses (Biton et al. 2006; Pichat et al. 2006).

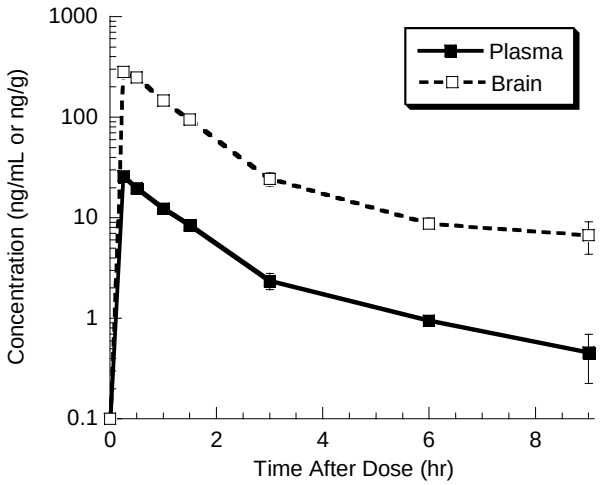

Figure 4 CNS levels of A-582941 in rodent. Brain:plasma distribution of A-582941 in mouse following a 1-μmol/kg (i.p.) dose ($n = 3$). The plasma C_{max} of 23 ng/mL is achieved within 15 min after dosing and the brain C_{max} (285 ng/g) is reached at by the first time point (20 min). The brain:plasma ratio is maintained at $\sim$10 for the 9-h duration of the experiment.

Animal Pharmacokinetics and Drug Metabolism

Consistent with its favorable logD and high permeability, A-582941 partitions into the CNS with high efficiency in both rat and mouse. Thus, brain levels of A-582941, following i.p. administration in mouse, reach C_{max} within 20 min at a level approximately 10-fold higher than the drug concentration in plasma (Fig. 4). This ratio is maintained relatively constantly for more than 8 h as the drug is eliminated with a half-life of about 2 h. A similar distribution was achieved following systemic administration of A-582941 in rat. Selective partitioning of A-582941 into brain could improve the therapeutic window for procognitive effects compared to side effects (e.g., cardiovascular or gastrointestinal [GI] effects) that might be mediated in the periphery, but requires that the excess

Table 1 Pharmacokinetic properties of A-582941

Species	i.v. administration				p.o. administration		
	Dose (μmol/kg)	$t_{1/2}$ (h)	V_{ss} (L/kg)	CL_p (L/h/kg)	Dose (μmol/kg)	C_{max} (ng/mL)	F (%)
Mouse	1.0	1.4	11.4	7.9	1.0	18	~100
Rat	6.2	1.5	9.2	4.7	6.2	114	90
Dog	0.5	1.4	7.9	5.3	3.0	79	22
Monkey	0.5	2.0	3.9	1.6	3.0	39	50

Vss, steady-state volume of distribution; CLp; plasma clearance; F, bioavailability after p.o. administration.

of drug in the brain is available for receptor activation (van de Waterbeemd et al. 2001). For passive diffusion across the blood–brain barrier, the laws of mass action dictate that "free" drug concentration in the brain (the fraction not partitioned into tissue or otherwise made unavailable for receptor activation) will not exceed that in plasma (Martin 2004; van de Waterbeemd et al. 2001). In the case of A-582941, drug concentrations in rat brain extracellular fluid (ECF) were estimated in conjunction with the determination of ACh release by microdialysis (Fig. 3B). Levels of A-582941 in dialysate from a probe in the medial prefrontal cortex peaked within 30–60 min following systemic administration of drug (3000 nmol/kg, i.p.). Based upon probe recovery measured *in vitro*, the drug concentration in ECF was estimated as 800–1200 ng/mL, in accord with whole-brain measurement of 980 ng/g calculated for this dose. Although *in vitro* calibration entails limitations for precise measurement *in vivo* (Stenken 1999), such estimates tend to underestimate brain levels due to greater resistance to diffusion of the analyte through brain tissue. We conclude that the free concentration of A-582941 in brain extracellular fluid is significantly (as much as 10-fold) higher than the plasma concentration, suggesting involvement of an active transport system that concentrates A-582941 in the brain relative to plasma.

A-582941 exhibits acceptable pharmacokinetic behavior in rodents, dog, and monkey (Table 1). In mouse and rat, the compound is well absorbed following oral administration, as evidenced by the very high oral bioavailability (~100% and 90%, respectively). The much lower bioavailability in dog is in accord with a much higher rate of metabolism (*vide infra*) in this species, and likely reflects a larger contribution of first-pass metabolism. Consistent with its lipophilic character, A-582941 is characterized by very large volumes of distribution (4–11 L/kg) and correspondingly high clearance values.

Metabolic conversion of A-582941 by liver microsomes or hepatocytes *in vitro* was highly species-dependent, with the rate of turnover fastest in dog, intermediate in rodent and monkey, and slowest in human (Fig. 5A). In each case, a single major metabolite tentatively identified as the N-oxide, **6** (Fig. 6), by MS-MS (stereochemistry undetermined) was observed.

To identify the oxidative enzymes involved in metabolism of A-582941, its turnover was evaluated across a panel of recombinantly expressed human cytochrome P450 enzymes (supersomes) (Brandon et al. 2003), indicating that multiple CYP isoforms (Fig. 5B) can contribute to oxidation of A-582941. Among cytochrome P450s, CYP2D6 causes the most rapid turnover, although significant oxidation by CYP1A2, CYP2A6, and CYP2C8 among others, implies that metabolism of A-582941 is unlikely to be strongly affected by drug–drug interactions. Flavin-containing mono-oxygenases, especially FMO1, may also contribute, particularly in dog and rodents that express this enzyme at high levels in the liver.

A radiotracer study in rat was used to investigate the metabolism of A-582941 *in vivo*. Following oral administration, [^{3}H]A-582941 was rapidly absorbed, consistent with its high bioavailability as described above. Circulating components included A-582941, the N-oxide, **6**, and a very polar metabolite assumed to be tritiated water. The mean total recovery of radioactivity at 24 h postdose was 80% (p.o.) and 85% (i.v.) of the dose, suggesting that elimination of radioactivity was rapid, with maximum elimination occurring within 24 h. Urine and feces accounted for 58–61% and 22–24% of the administered dose, respectively, indicating that the major route of elimination of drug-derived radioactivity was via the urine. The compound is substantially metabolized, with only 16–18% unchanged drug in the urine and 5–6% in feces. The N-oxide, **6**, is found as a major component in the urine (17–23%), but only in trace amounts (0–1%) in feces. The apparent formation of tritiated water suggests that loss of the methyl group of A-582941 might occur. This could involve direct oxidation of the methyl group and fragmentation of the hydroxymethylamine, or alternatively, elimination of hydroxide from the N-oxide, **6**, and hydrolysis of the resulting imine. Both routes ultimately lead to formation of **7** (Fig. 6), but that product cannot be detected in the radiotracer study. A separate investigation with unlabeled A-582941 revealed that circulating levels of **7** are quite low (1% of parent levels in brain, 3% in plasma), suggesting that demethylation is not a major route of metabolism for A-582941. We cannot, however, rule out a process that couples demethylation with efficient elimination (conjugation, etc.).

In Vivo Behavioral Pharmacology

A-582941 was evaluated across a battery of behavioral assays in rodent and nonhuman primate models that assess

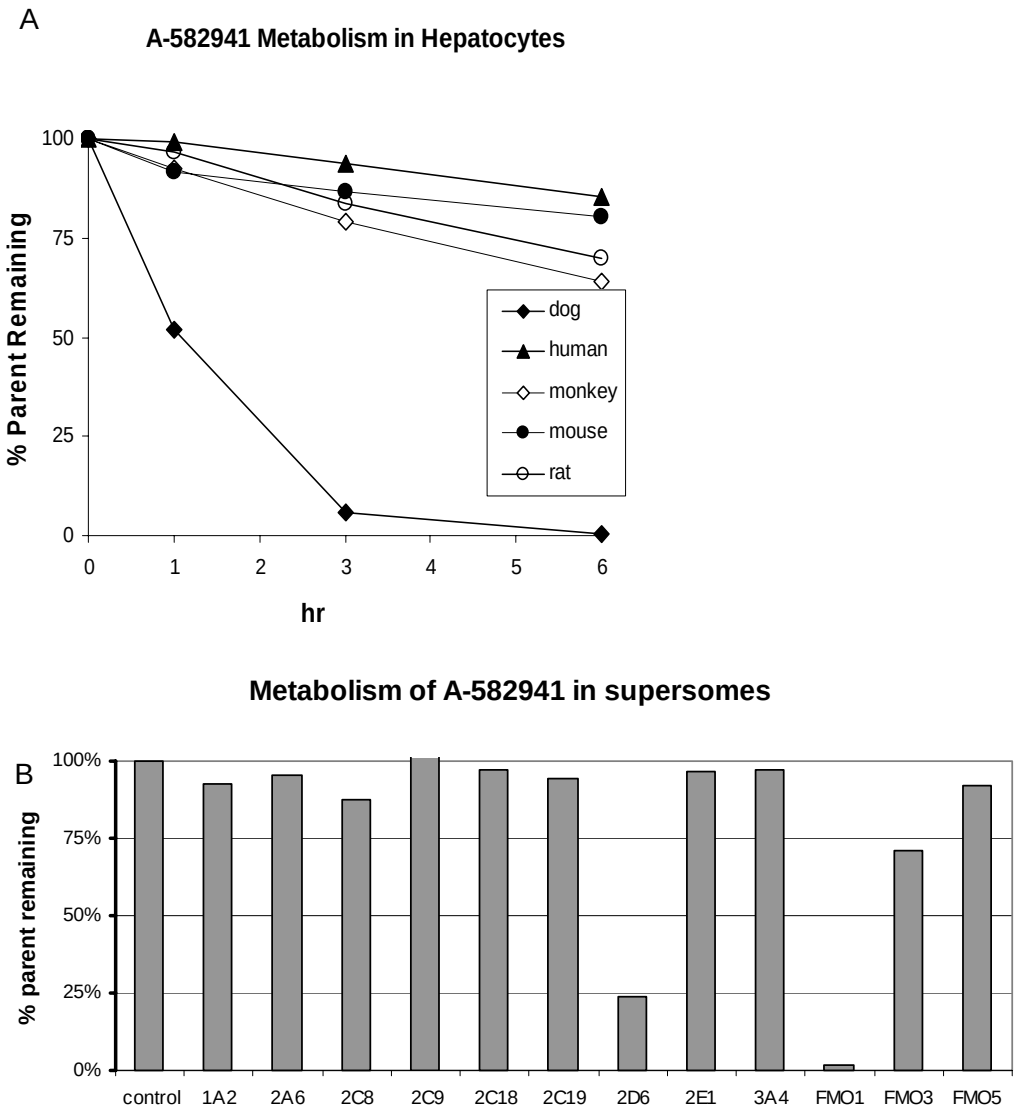

Figure 5 (A) Time course for *in vitro* metabolism of [^{3}H]A-582941 in liver hepatocytes. The starting concentration of A-582941 was 1.0 μM. (B) Turnover of A-582941 across a panel of CYP and FMO supersomes.

cognitive performance (Bitner et al. 2007). A summary of *in vivo* efficacy data and corresponding efficacious plasma concentrations is presented in figure 7 and table 2.

Social Recognition in Rats

A-582941 was efficacious in the social recognition test, a model of short-term memory on the basis of olfactory cues. An adult rat was presented with an unfamiliar juvenile for a 5-min session, and the fraction of time the adult spends on investigation of the juvenile (sniffing, grooming, and close following) was recorded (T1). The juvenile was then removed from the cage, and the adult animal was treated with A-582941 or saline. After 2 h, the same juvenile was reintroduced, and the investigative behavior of the adult was monitored for the second session (T2). Whereas the saline-treated adults exhibit little recognition of the juvenile during the second session, as evidenced by investigation times nearly equal to the first session, A-582941 caused a dose-related reduction in exploration time for the second session compared to the first session, indicating improved recognition of the juvenile by the adult (Fig. 7Ai) (Bitner et al. 2007). The effect was specific for the original juvenile animal, for if a different juvenile was introduced, the adult spent as much time investigating as for the initial session. To assess whether

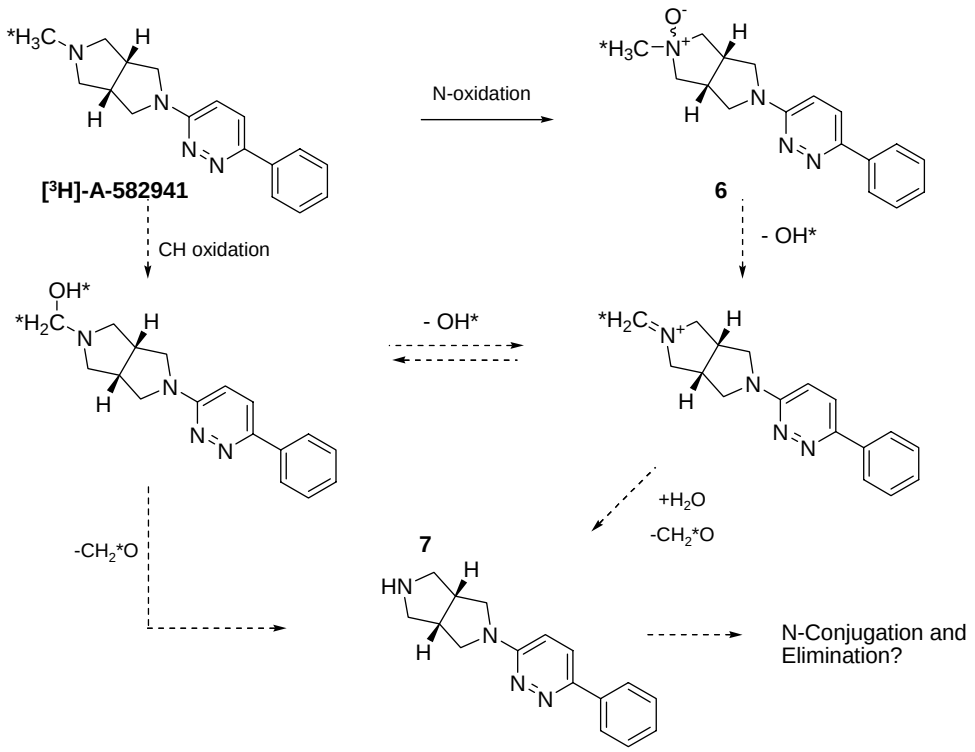

Figure 6 Metabolism of A-582941. Oxidation to the N-oxide, **6**, is the predominant route for metabolism in microsomes and hepatocytes (all species). The formation of tritiated water from [^{3}H]A-582941 *in vivo* suggests that N-demethylation to **7** may occur. The secondary amine could undergo rapid conjugation and/or elimination.

this efficacy could be maintained following sustained exposure, rats were implanted with miniosmotic pumps to deliver steady-state plasma levels of A-582941 for 7 days. The mean plasma concentration achieved in A-582941-treated groups was 3.1 $\pm$ 0.3 ng/mL ($n = 9$). On the 7th day, the animals were evaluated in the social recognition test. As shown in figure 7B, A-582941-treated animals retained efficacy as reflected by the reduction in ratio between the first to second contact. The steady-state plasma levels from the A-582941 sustained infusion were similar to the plasma levels ($C_{max} = 4.8$ ng/mL, extrapolated from dosing a separate group of animals at 2.0 μmol/kg, i.p.) achieved with a single acute injection of the 0.1-μmol/kg efficacious dose.

Delayed Matching-To-Sample Test in Primates

To assess effects on executive cognitive function of short-term memory, the delayed-matching-to-sample (DMTS) model in primates was employed. In this task, young Rhesus monkeys are presented with a light stimulus of a particular color. After a delay interval, the animal is presented with two stimuli colors, one corresponding to the original stimulus and on another, different color. Correct identification of the target color is rewarded. Performance on this task is a function of the delay interval, declining nearly to chance levels at "long" delays (the delay interval is adjusted for individual animals depending on their abilities in this task, the long delay corresponds to baseline performance of 55–64% for these animals) (Buccafusco et al. 2007). Administration of A-582941 (3.0–100 nmol/kg, i.m.) produces a dose-related improvement in performance at long delay intervals (Fig. 7Aii), but not at short delay intervals. This may indicate that A-582941 augments short-term memory processing, but may be less effective in facilitating attentional processes that are more important at the short delays (Buccafusco et al. 2007). Based upon PK measurements in a satellite group of animals, the plasma level associated with a fully efficacious dose (10 nmol/kg, i.m.) was 5.9 ng/mL, similar to that for the rodent cognition models.

Inhibitory Avoidance (One Trial) in Mice

The inhibitory avoidance task, which uses a two-compartment step-through apparatus, measures the ability to remember a brief noxious stimulus such as foot shock. Briefly, mice placed in a lighted compartment of

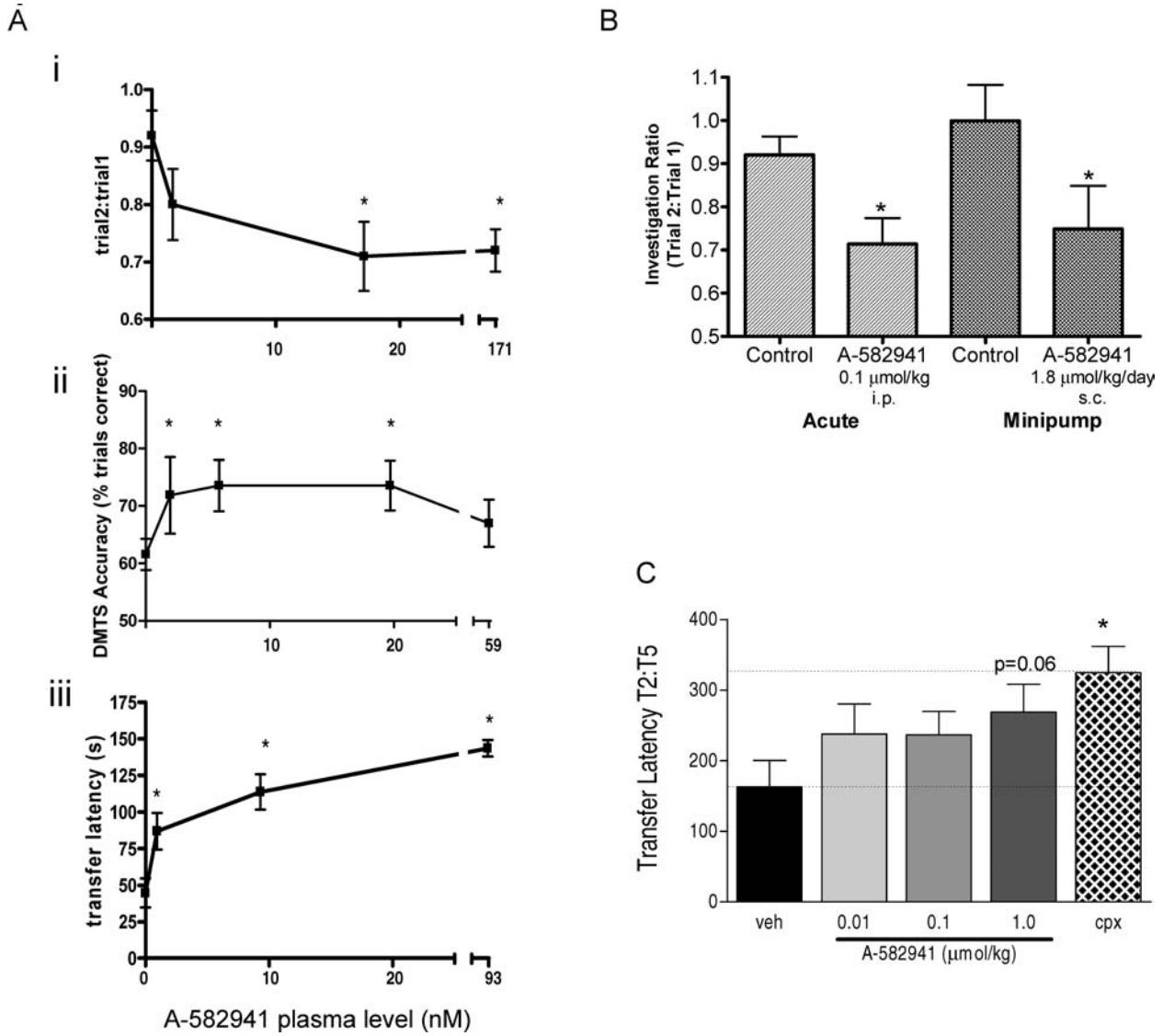

Figure 7 Procognitive efficacy of A-582941. (A) A-582941 is efficacious across different species and models at similar plasma exposures: (i) lowered trial2:trial1 investigation ratio indicating enhanced efficacy in the rat social recognition model; (ii) increased correct response rate at the long delay interval for monkeys in the DMTS task; and (iii) increased crossover latency in the 24-h test trial of the mouse inhibitory avoidance test, a measure of memory consolidation. Data are replotted from Buccafusco et al. (2007) (ii) and Bitner et al. (2007) (i and iii) with plasma exposure (extrapolated from dosing in a satellite group of animals) representing the C_{max} at the corresponding dose. The left-hand segment of each graph is on the same horizontal scale. (B) Efficacy of A-582941 in social recognition model following acute and sustained 7-day exposure. Recognition ratio was measured following acute treatment with A-582941 (i.p.) or after sustained exposure via s.c. osmotic minipumps for 7 days. The plasma level extrapolated from an i.p. dose of 1 μmol/kg in satellite mice corresponds to 4.7 ng/mL, whereas minipump exposure maintained steady-state plasma levels at 3 ng/mL. (C) Effect of A-582941 on the performance of spontaneously-hypertensive rat pups in the 5-trial inhibitory avoidance model. A trend toward efficacy is observed in the dose range 0.01–1.0 μmol/kg, s.c., but full efficacy is not achieved. Ciproxifan (cpx, 3 mg/kg) is the positive control in this assay.

the apparatus prefer stepping through into a dark compartment where a mild foot shock is delivered. Retention testing is conducted 24 h later with the animal again being placed in the lighted compartment (but with no drug treatment) and its latency to enter the dark side of the apparatus measured (no shock). Increasing entry latency is regarded as an index of memory. A-582941 (0.01, 0.10, and 1.00 μmol/kg, i.p.) administered 30 min prior to the shock training trial, evoked a dose-dependent increase in crossover latency during the retention testing

Table 2 Summary of efficacy of A-582941 in animal models of cognition

Cognitive domain	Model	Species	Effective dose(s) (μmol/kg)	Efficacy/dose (μmol/kg)	A-582941 plasma concentration ng/mL	nM
Working memory	Delayed match-to-sample (DMTS)	Monkey	0.003–0.100	+++/0.01	5.9	21.0
Short-term recognition memory	Social recognition	Rat	0.1–1.0 (acute) 1.8 μg/kg/day (chronic)	+++/0.10 +++/OMP	4.8 3.1	17.0 11.0
Memory consolidation	24-hr inhibitory avoidance	Mouse	0.01–1.00	+++/0.10	2.6	9.3
Sensory gating	Auditory evoked potential	Mouse	3.0–10.0 (acute)	++/3.0 (repeated dosing)	44.0	157.0
Response inhibition/ impulsivity	5-trial inhibitory avoidance	Spontaneously-hypertensive rat pup	0.1–1.0*	++/1.0	48.0	171.0

+++, 70–100%; ++, 30–70%; +, <30%; 0, inactive; efficacy relative to positive control used in the respective experiments; OMP, administration s.c. via osmotic minipump; testing after 7 days.
*$P = 0.06$ at highest dose.

phase (Fig. 7Aiii) (Bitner et al. 2007). Pretreatment with mecamylamine, but not DHβE, attenuated A-582941-evoked crossover latency, indicating that the effect was mediated by α7 nAChRs. Plasma levels of A-582941 associated with the 0.1-μmol/kg dose, which achieves full efficacy, average 2.6 ng/mL (C_{max}), in line with drug exposures that are effective in the rat social recognition and monkey DMTS models.

Sensory Gating (N40) in Rats and DBA2 Mice

The effects of A-582941 on sensory gating deficits were assessed in both rat and DBA2 mouse. Normal sensory gating (N40 or P50) consists of an attenuation of the EEG amplitude evoked by the second (test) versus the first (condition) auditory stimulus. An increase in the ratio of test versus condition response or T:C ratio is an indication of sensory gating impairment, and has been observed in schizophrenia and Alzheimer's disease (Adler et al. 1998; Jessen et al. 2001). The sensory gating deficit evoked by i.c.v. administration of MLA (30 μg) in rat was reversed by preadministration with A-582941 (10 μmol/kg, i.p.), suggesting that the MLA-induced deficit is mediated through the α7 nAChRs (Bitner et al. 2007). In conscious DBA/2J mouse, a strain known to exhibit a genetic deficit in N40 gating (Radek et al. 2006), A-582941 (3 and 10 μmol/kg, i.p.) significantly improved sensory gating in these animals. Further, efficacy was retained following repeated injection of A-582941 (3 μmol/kg, i.p., for 5 consecutive days followed by testing on the 5th day), suggesting that, like in the social recognition model, efficacy in the N40 gating model can be maintained following repeated dosing (Bitner et al. 2007). Somewhat higher doses were required for efficacy in the gating model, and plasma levels at the end of the 5-day repeated dose study averaged 44 ng/mL, approximately 10-fold higher than exposures that are efficacious in the other cognition models.

5-Trial Passive Avoidance in Spontaneously Hypertensive Rat (SHR) Pups

A-582941 was also tested in SHR pups, trained in a 5-trial, repeated acquisition response assay with components of attention and impulsivity as previously described (Fox et al. 2002). Briefly, pups were trained to avoid a mild foot shock (0.1 mA, 1-second duration) delivered upon movement from a brightly illuminated to a darkened compartment (which they normally prefer) of the inhibitory avoidance system. After the first trial, the pup was removed and returned to its home cage and the transfer latency was noted. After approximately 60 second, the same pup was again placed in the brightly illuminated compartment, and the training process was repeated; a total of 5 trials were conducted in this manner. A-582941 or saline vehicle was injected s.c. 30 min prior to the first trial. A-582941 at doses ranging from 0.1–1.0 μmol/kg modestly enhanced performance in the model as evidenced by the increased cumulative transfer latencies relative to vehicle-treated controls.

However, the overall maximal effect (65% at 1.0 μmol/kg) is lower than that observed with the histamine H_3 antagonist, ciproxifan (3.0 mg/kg), which was used as a positive control (100%) in this experiment.

Collectively, these studies demonstrate that A-582941 exhibits broad-spectrum efficacy across various domains of cognition including working memory, short-term recognition memory, long-term memory consolidation, and preattentional sensory gating. With respect to cognitive domains, A-582941 appears to be especially effective in tasks that involve learning and memory (monkey DMTS at long delay, rat social recognition, and mouse inhibitory avoidance). Interestingly, the procognitive effects of A-582941 in these models were manifested at similar plasma exposures, approximately 3–6 ng/mL (10–20 nM) across several models and species (Fig. 7A). These plasma levels correspond closely to the binding affinity for A-582941 at the α7 nAChR, and are approximately 200-fold lower than the EC_{50} for ion channel opening *in vitro*, indicating that signaling can occur at agonist concentrations well below those required for a macroscopic current in oocytes, measured as the synchronous opening of many channels elicited by rapid application of agonist. Since the level of A-582941 in brain may be as much as 10-fold higher than in plasma, the effective concentration of A-582941 at the α7 nAChR is estimated to be in the 100–200 nM range, in excellent agreement with concentrations that cause increased phosphorylation of ERK1/2 *in vitro* (EC_{50} = 95 nM). Thus, the brain levels of A-582941 required to elicit effects *in vivo* lie at the low end of the concentration–response curve defined *in vitro* using rapid agonist application. Nevertheless, low concentrations of agonist can elicit a small, but sustained, current by stimulating the opening of a small percentage of the α7 nAChR. Individual receptors may be stimulated, desensitized, and recovered with normal dynamics, while the size of the pool of open channels remains relatively stable at any one time. In this way, *in vivo* efficacy of agonists administered systemically can be reconciled with the rapid receptor desensitization identified *in vitro*. *In vivo* effects may be mediated by depolarization, Ca^{2+} influx, or both.

Secondary Pharmacodynamic Profile

Well-known side effects of nicotinic receptor agonists at high doses include changes in heart rate and arterial pressure, GI effects, including nausea and emesis, and CNS effects, such as deficits in motor coordination, hypothermia, and seizure activity. The cardiovascular effects of A-582941 were evaluated in anesthetized dog. Intravenous infusion of increasing concentrations of A-582941 revealed a benign hemodynamic profile, with only a modest (9%) increase in heart contractility that plateaued at higher doses. No significant changes in heart rate, pressure, or vascular resistance were observed at plasma exposures up to 899 ng/mL, approximately 150-fold above efficacious levels. A modest increase in the corrected QT interval was observed, reaching 7% at the highest exposure (899 ng/mL = 3200 nM). Increases in QT interval can result from effects on ion channels that regulate cardiac repolarization, and blockade of the human ether-a-go-go (hERG) channel has been demonstrated to be a key factor for many drugs that cause QT prolongation (Roden 2004). A-582941 displaced [^{3}H]dofetilide from hERG-transfected HEK-293 cells (Diaz et al. 2004) with a K_i value of 4300 nmol. Functionally, using patch clamp methods, A-582941 was shown to block hERG channels with IC_{50} = 4800 nmol. A-582941 also lengthens duration of the action potential in canine Purkinje fibers in a concentration-dependent fashion (Reinhart et al. 2005), reaching 11% at 1700 nmol and 32% at 17,000 nmol. These *in vitro* data are consistent with the modest increase in QT interval observed in anesthetized dogs at plasma concentrations that are at least 100-fold higher than those required for procognitive efficacy. It is worth noting that activity at hERG also was observed as a liability in the quinuclidine α7 ligand PNU-282987, but in that instance, it proved possible to identify analogs that exhibited diminished hERG blockade while maintaining α7 activity (Walker et al. 2006).

nAChR agonists, including nicotine and the $\alpha4\beta2$ partial agonist varenicline, are known to cause nausea and emesis as a key adverse event in humans (Haro and Drucker-Colin 2004; Jorenby et al. 2006; Lemay et al. 2003; Silver et al. 2001; Tonstad et al. 2006). Such effects have been attributed to activation of $\alpha3\beta4^*$ nAChRs that are highly expressed in the autonomic ganglia (Jain 2004; Ji et al. 2007; Jones and Dunlop 2007), but the possible role of α7 agonism in GI effects has not been clearly delineated. Emesis testing in ferrets has been employed as a preclinical model for predicting the liability for GI effects in humans (Osinsky et al. 2003). A-582941 was administered to ferrets (6 per group) at doses of 3, 10, and 30 μmol/kg, i.p. Emesis was seen only at the highest dose, in 1 of 6 animals. Plasma concentrations for the high-dose group (taken 30 min after drug administration) averaged 1180 ng/mL (4200 nM), representing a ~200-fold multiple over the efficacious range. This exposure corresponds to the EC_{50} for ionotropic activation of α7 nAChRs *in vitro*, and we cannot rule out involvement of α7 in the emetic response. On the other hand, it should be noted that A-582941 also activates $5HT_3$ channels at this concentration, and some $5HT_3$ agonists produce a proemetic response in preclinical models

(Dukat et al. 2000; Kamato et al. 1993). Investigation of this mechanistic question by use of selective antagonists would require still higher doses of A-582941 to establish a robust emesis response, and was not pursued.

Toxicology Profile

A-582941 was nonmutagenic in the Ames reverse mutation assay in five bacterial systems (*Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2uvrA), even with metabolic activation by microsomal S9 fraction from rat. Likewise, A-582941 was nonclastogenic in the micronucleus assay. Taken together, these results indicate that A-582941 is not a direct-acting gene toxin.

While evidence has emerged that downstream activation of pERK1/2 in the CNS by α7 agonists, including A-582941, is crucial to their procognitive effects, activation of this pathway in the periphery can be associated with cell proliferation and carcinogenesis (Schuller et al. 2000; Wong et al. 2007). Moreover, α7 nAChRs have been implicated in angiogenesis, a process that could contribute to tumor growth (Heeschen et al. 2001; Minna 2003; West et al. 2003). Therefore, several studies were designed to interrogate the potential of A-582941 to contribute to carcinogenesis.

To determine whether administration of A-582941 induced gene expression changes that are linked to proliferative and other toxicities, rats were dosed with A-582941 (1 and 30 μmol/kg, i.p.) for 3 days. Even at these high exposures (corresponding to 10- and 300-fold above a procognitive dose), relatively few gene changes were noted by microarray analysis of tissue from kidney and liver. Drug matrix analysis of those gene changes produced no matches with known toxins, indicating that A-582941 does not activate pathways characteristic of any known hepatotoxic, nephrotoxic, or carcinogenic agents. The relatively modest set of gene expression changes could not be correlated with any known mechanisms of toxicity.

In the periphery, α7 nAChRs are highly expressed in keratinocytes, and appear to be involved in differentiation of the epithelial layer (Arredondo et al. 2002). Thus, α7 knockout mice exhibit a thickened, multilayer epidermis characteristic of delayed epidermal turnover. Additionally, studies related to the endogenous α7 nAChR modulator SLURP have implicated α7 nAChR in a human skin disorder (Chimienti et al. 2003; Eckl et al. 2003). Therefore, the influence of α7 activation on keratinocyte cell proliferation was examined. Exposure of cultured human keratinocytes to A-582941 (100 and 10,000 nmol) produced no detectable proliferation under conditions where serum/growth factor supplements evoked significant proliferative events (Fig. 8A).

To further explore the potential of A-582941 to promote tumor growth, the compound was applied to shaved skin patches on the backs of SENCAR (sensitive to carcinogens) mice every 2 days for a period of 2 weeks. Exposure measured by analysis of skin samples taken 1 h after dosing a satellite group of animals averaged 6 ng/g ($\sim$21 nM). In this assay, A-582941 produced only a mild irritation of the skin, with no histologic evidence of epidermal hyperplasia and increased proliferation of basal keratinocytes as assessed by immunohistochemical detection of BrdU. (Fig. 8B)

In animal experiments, A-582941 was well tolerated at exposures greater than those required for efficacy in cognition models. Mice dosed at 10 μmol/kg (i.p.) exhibited no significant difference from vehicle-treated animals in a mini-Irwin assay, representing a margin of 100-fold relative to efficacy in the inhibitory avoidance test. In rats, single oral dosages of 400 mg/kg (1400 μmol/kg) induced clinical signs indicating CNS activity, such as tremor and spasms. A dose of 800 mg/kg, p.o., was lethal within 10 min. Rats dosed at 50 mg/kg (180 μmol/kg, p.o.) QD for up to 10 days exhibited clinical signs of general intolerance and a slight body weight loss. In liver, minimal centrilobular hypertrophy was noted associated with slightly increased mitotic figures. The time-averaged plasma exposure at this dose (area under the curve [AUC] = 6500 ng-h/mL) represented a >50-fold margin above the C_{max} for efficacy in the rat social recognition model, indicating an acceptable safety margin of A-582941 relative to systemic toxicity.

Conclusions

A-582941 was selected from a structurally novel (non-quinuclidine) class of α7-selective agonists with drug-like physicochemical properties and excellent CNS penetration. The compound exhibited good efficacy across a range of rodent and primate cognition models thought to reflect different domains involved in memory and learning, including attentional processes, memory consolidation, and executive function. Plasma exposures at efficacious doses closely approximated the binding affinity of A-582941 for the α7 receptor ($\sim$11 nM) and were well below levels required to induce ionotropic activity for this receptor *in vitro* (2400 nM). Brain levels of A-582941 were approximately 10-fold higher than plasma exposures, but were still low relative to the agonist EC_{50} for ion channel opening. On the other hand, those levels correlated well with the potency of A-582941 toward increasing ERK1/2 phosphorylation *in*

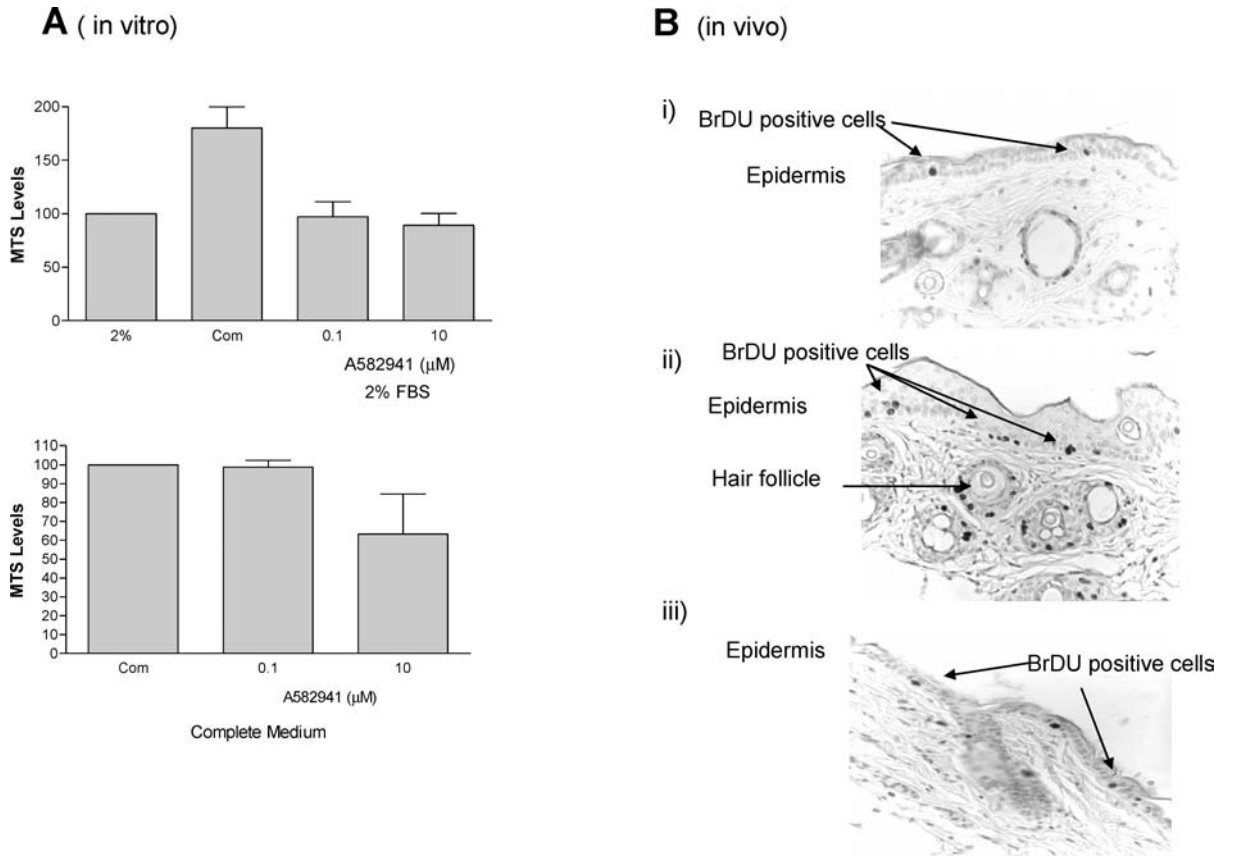

Figure 8 Lack of effect of A-582941 on cell growth and proliferation on keratinocytes *in vitro* and *in vivo*. (A) *In vitro* effect of A-582941 (100 and 10,000 nM) on proliferation of cultured keratinocytes in basic medium: (i) without growth factors and (ii) in complete medium containing growth factors. Cells were exposed to A-582941 for 9 days, with medium refreshed every 3–4 days. (B) Histologic sections of skin from SENCAR mice sacrificed after treatment with BrdU reagent (Zymed® Laboratories, San Francisco, CA, USA; 1 mL/100 g body weight, i.p., 2 h before sacrifice): (i) vehicle-treated mice; (ii) mice treated with TPA (12-O-tetradecanoylphorbol-13-acetate, 1.7 nmol) as positive control. Note the thickening of the epidermis (epidermal hyperplasia) and the increase in the number of BrdU-labeled basal keratinocytes; (iii) A-582941-treated mice (1000 nmol) showing no evidence of epidermal changes and few BrdU-labeled basal keratinocytes. BrdU immunohistochemistry with hematoxylin counterstain (magnification 200×).

vitro (95 nmol), a downstream consequence of α7 activation that, along with CREB activation, is also observed at these doses *in vivo*. It is possible that low levels of α7 channel opening are all that are required to trigger these downstream events. It is also possible that some other (metabotropic) signaling path, possibly mediated by Ca^{2+}, is available to the α7 receptor at these low agonist concentrations. In any case, activation of the α7 nAChR by A-582941 evokes downstream changes in pERK and CREB that mirror efficacy in rodent and primate models of learning and memory.

The predominant adverse effects of A-582941, observed at doses substantially above those that are efficacious in cognition models, include clinical signs of CNS activity (tremors). Emesis and CV effects are of relatively minor concern. Although moderate QT_c prolongation is observed with A-582941 in dog at high plasma exposures, it does not appear to be α7 related. Investigation of potential carcinogenic effects produced no significant findings to suggest any α7-related toxicities.

A-582941 therefore adds to the preclinical validation of the α7 nAChR for treatment of cognitive deficits. Its broad-spectrum efficacy, excellent tolerability, and relatively low potential for toxicity have made it a valuable tool compound for evaluation of the α7 nAChR platform, and in helping to establish its potential as a target for drug development.

Addendum

Compound names associated with the abbreviations used in the text are as follows: A-585539, (*1S,4S*)-2,2-

Dimethyl-5-(6-phenylpyridazin-3-yl0-5-aza-2-azoniabicyclo[2.2.1]heptane; ABBF, (*R*)-*N*-1-azabicyclo[2.2.2]oct-3-yl)-7-(2-methoxyphenyl)-1-benzofuran-2-carboxamide; AR-R 17779, (*S*)-Spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one]; BRL-46394, (*endo*-*N*-(9-methyl-9-azabicyclo[3.3.l]non-3-yl)-l-methyl-(*1H*)-indazole-3-carboxamide; GTS-21, (*E*)-3-(3-(2,4-dimethoxybenzylidene)-3,4,5,6-tetrahydropyridin-2-yl)pyridine; PASB-OFP, (*R*)-5′-phenylspiro[1-azabicyclo[2.2.2]octane-3,3′(3′*H*)-furo[2,3-b]pyridine]; PHA-543613, (*R*)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-furo[2,3-c]pyridine-5-carboxamide; PNU-120596, 1-(5-chloro-2,4-dimethoxyphenyl)-3-(5-methylisoxazol-3-yl)urea; PNU-282987, (*R*)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-4-chlorobenzamide; SSR-180711, 4-bromophenyl 1,4-diazabicyclo[3.2.2]nonane-4-carboxylate; W-56203, (*R*)-3′-(3-methylbenzo[*b*]thiophen-5-yl)spiro-[1-azabicyclo[2,2,2]-octane-3,5′-oxazolidin]-2′-one.

Conflict of Interest

All authors are employees of Abbott Laboratories, the developer of A-582941.

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